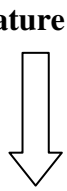
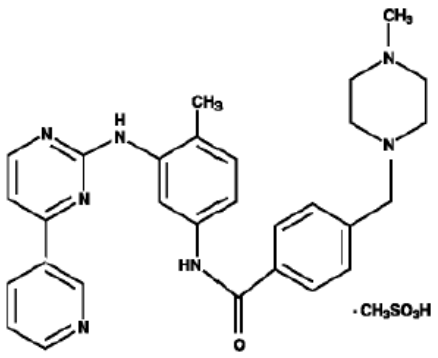
1. **Work plan**


Literature search
pKa value of the Molecule
Solubility of Molecule
Selection of Mobile Phase and Column
Methods optimization for HPLC
Validation as per Guidelines

Imatinib Mesylate:

(4-[(4-methylpiperazin-1-yl)methyl]-N-[4-methyl-3-[(4-pyridin-3-ylpyrimidin-2-yl)amino]phenyl]benzamide)



Molecular Formula: $C_{29}H_{31}N_7O.CH_4SO_3$

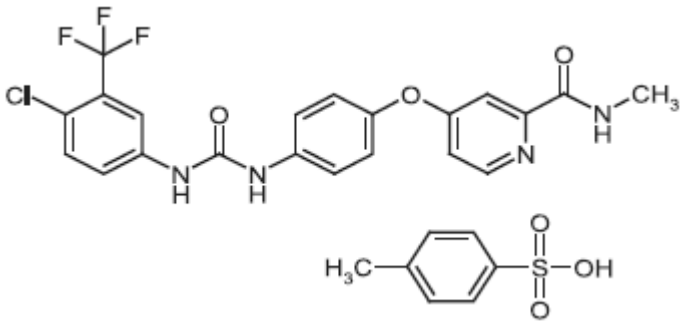
Molecular Weight: 589.7

Solubility: soluble in aqueous buffers ≤ pH 5.5

Imatinib (originally STI571) is a drug used to treat certain types of cancer. It is currently marketed by Novartis as Gleevec (USA) or Glivec (Europe/Australia/Latin America) as its mesylate salt, imatinib mesilate (INN). It is used in treating chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs) and some other diseases. By 2011, Gleevec has been FDA approved to treat ten different cancers. In CML, the tyrosine kinase enzyme ABL is stuck in its activated form; imatinib binds to the site of tyrosine kinase activity, and prevents its activity.

Imatinib, formerly referred to as STI571, is an inhibitor of specific protein tyrosine kinases that was targeted to the platelet-derived growth factor (PDGF) receptor. It was found to inhibit the constitutively active fusion product arising from the Philadelphia chromosome of chronic myelogenous leukemia and C-kit, which is overexpressed in gastrointestinal stronal tumors. Studies of imatinib in other tumors that express c-kit or the PDGF receptor are under way. Imatinib was approved by the food and drug administraton (FDA) in may 2001 for the treatment of CML, that is refractory to interferon therapy and in februrary 2002 for the treatment of gastrointestinal stromal tumors.[28]

Sorafinib Tosylat:



Molecular Formula: $C_{21}H_{16}ClF_3N_4O_3$ x $C_7H_8O_3S$

Molecular Weight: 637.0

Solubility: practically insoluble in aqueous media, slightly soluble in ethanol and soluble in PEG 400.

Sorafenib is used to treat advanced renal cell carcinoma (a type of cancer that begins in the kidneys). Sorafenib is also used to treat unresectable hepatocellular carcinoma (a type of liver cancer that cannot be treated with surgery). Sorafenib is in a class of medications called multikinase inhibitors. It works by slowing the spread of cancer cells.[17]

June 11, 2007 (Chicago) — Onyx Pharmaceuticals' sorafenib (*Nexavar*) is the first effective systemic treatment for advanced liver cancer. Presenting here at the 43rd annual meeting of the American Society of Clinical Oncology, researchers showed that the targeted multikinase inhibitor extends survival by 44%. "This improvement is dramatic," Philip Johnson, MD, from the Institute for Cancer Studies at the University of Birmingham, in the United Kingdom, said during a discussion period following the presentation. "This is the dawn of a new era for hepatocellular carcinoma therapy," he said, but he also cautioned that just like at the dawn of a new day, "we can't necessarily see everything until the light shines."

During a press briefing outlining the findings, session moderator William Blackstock, MD, from the Wake Forest University School of Medicine, in Winston-Salem, North Carolina, was very enthusiastic about the new trial. "This is an example of a study that couldn't have been done in

the United States," Dr. Blackstock told Medscape. "Cancer patients here would never have accepted a placebo arm like the one in this trial. They expect to be treated."

He said that in addition to providing a first-line option for difficult-to-treat patients, these findings out of Spain will revolutionize future liver cancer clinical trials. "I can't tell you what the design will be for these trials," Dr. Blackstock said, "but I can tell you what the control will be — sorafenib."[28]

4.1 METHODOLOGY

Materials and Method

High performance liquid chromatography/ Ultra performance liquid chromatography equipped with gradient pump and photo diode array detector and Mass Detector. Anti cancer drug will be procured from local market. Reference standard of the anti-cancer drugs and related impurities will be studied. Analytical grade reagents and HPLC/UPLC Grade solvents to be procured local market. The drug substances will be analyses with respect to the separation and quantitation of its related impurities using liquid chromatography.

Aims and Objective of the Proposed Research

- ✓ To develop selective and methods which are simple reliable workable and economical under routine conditions.
- ✓ To evaluate and study the stability indicating nature of the method.[29]
- ✓ Validation of the analytical methods. .[30]
- ✓ To apply developed analytical methods to various formulations.
- ✓ EMEA guide line for genotoxic impurity.[31]

PHARMACEUTICAL ANALYTICAL TECHNIQUES

Pharmaceutical analysis deals with the scientific and technical aspects of measurement of compositional and constitutional features of the sample. It can be broadly divided into

- ➢ Qualitative Analysis(Identification)
- ➢ Quantitative Analysis(Estimation)

Qualitative Analysis reveals the identity of species i.e. the identification of compounds and impurities in the sample.

The various steps involved in a typical quantitative analysis are

- Chemical nature of the sample
- Obtain adequate amount of sample
- Selection of method
- Preparation of laboratory sample
- Number of samples to be analyzed
- Elimination of possible interferences
- Measurement of analyte
- Estimation of the reliability of the results(validation)
- The importance of newer analytical methods

Drugs analysis means identification, characterization and determination of impurities in drug substances. The number of drugs introduced into the market has been increasing at an alarming rate.

These drugs may be new entities in the market or structural modifications of the existing drug. Newer analytical methods are developed for these drug combinations because of the following reasons:

- The drug or drug combination may not be official in any pharmacopoeia.
- The literature search may not reveal an analytical method for the drug or its combinations.
- Analytical methods may not be available for the drug combination due to the interference caused by excipients.
- Newer methods are also recommended by Research Institutions.
- Quality control Department in Industries Approved Testing Laboratories.

ANALYTICAL METHOD DEVELOPMENT

HPLC/UPLC method development and validation is important for the analyzing of impurities in any Drug substances. Whatever method is used for quantitation or identification of impurities, the method should be a validated one. The method must be able to detect or quantitate the particular impurities in presence of Drug substances. Before starting any method development

one has to have knowledge about the information of the nature of sample, define separation goals, number of compounds present, chemical structures, molecular weights, pKa values, solubility and UV spectrum of the compounds. Perhaps maximum method development involves the trial and error procedures.

Selection of mobile phase

The selection of the mobile phase mainly based on the solubility and polarity of the compound. Usually, in RP-HPL/UPLC method water and organic solvents are used as the mobile phase. In NP-HPLC method non polar solvents like Hexane and THF were used. If the sample contains ionic or ionizable compounds, then use of a buffered mobile phase to ensure the reproducible results. Under unfavorable circumstances, pH changes as little as 0.1 pH units can have a significant effect on the separation. On the other hand properly used buffer allows controlling the pH easily. Buffer works best at the pKa values of its acid. At this pH, the concentration of the acidic form and the basic form of the buffering species are equal, and the buffering capacity is maximum. Phosphate has three pKa values in the range of interest for silica based chromatography at pH 2, pH 7 and pH 12.32. The pKa of acidic buffer is 4.75.

Selection of buffer

In reversed phase chromatography mobile phase pH values are usually between 2.0 and 7.5. Buffers are needed when an analyte is ionisable under reversed phase conditions or the sample solution is outside this pH range. Analyte ionisable under reverse phase conditions often have amine or acid functional group with pKa between 1.0 and11.0. A correctly chosen buffer pH will ensure that the ionisable functional group is in a single form, whether ionic or neutral. If the sample solution is at pH damaging to the column, the buffer will quickly bring the pH of the injected solution to a less harmful pH.

Selection of Buffer pH

pH is another factor in the resolution that will affect the selectivity of the separation in reversed-phase HPLC/UPLC. In reverse-phase chromatography sample retention (K') increases when the analyte is more hydrophobic (non-polar). Sample retention (K') decreases when the analyte is more hydrophilic (polar). Thus when an acid or base is undergoes ionization it becomes more

hydrophilic and less interacting with column binding sites. When the pH value of the mobile phase equal to the pKa value of the analyte, it is said to be half ionized, i.e. the concentration of the ionized and unionized species are equal. As mostly all of the pH caused changes in the retention occur within ± 2.0 pH unit of the pKa value, it is best to adjust the mobile phase to pH value at least ± 2.0 pH unit above or below the pKa to ensure practically 100% unionization of analyte for retention purpose.

Selection of column

The HPLC/UPLC column is the heart of the method, critical performing the separation. The column must possess the selectivity, efficiency and reproducibility to provide good separation. Commonly used reversed phases are cross linking the Si-OH groups with alkyl chains like, C8 (octylsilane), C18 (octadecyl silane) and nitrile groups (CN), phenyl groups (-C6H6) and amino groups (-NH2). They are chemically different bounded phases and demonstrate significant changes in the selectivity using the same mobile phase.

1. Length and diameter of the column.
2. Packing material.
3. Shape of the particles.
4. Size of the particles.
5. % of carbon loading.
6. Pore volume.
7. Surface area.
8. End capping.

Selection of Column temperature

Temperature variation over the course of a day has quite significant effect on HPLC/UPLC separations. This can even occur in air conditioned rooms. While temperature is a variable that can affect the selectivity, its effect is relatively small. Always it is preferable to optimize the chromatographic conditions with column temperature as ambient. The increase in column temperature generally will result in reduction in peak asymmetry and peak retentions. When found necessary, the column temperatures between 30ºC and 80ºC shall be adopted.

Selection of flow rate

Flow rate, more for isocratic than gradient separation, can sometimes be useful and readily utilized to increase the resolution, although its effect is very modest.

Flow rate shall be selected bases on the following data.

- Retention times.
- Column back pressures
- Separation of impurities.
- Peak symmetries.

Preferably the flow rate shall be not more than 2.5 ml/min. check the ruggedness of the method by varying the flow rate by ± 0.2 ml from the selected flow rate. Select the flow rate which gives least retention tomes, good peak symmetries, least back pressures and better separation of impurities from each other and from API peak.

Selection of Solvent delivery system

Chromatographic separation with a single eluent (isocratic elution) i.e.: All the constituents of the mobile phase is mixed and pumped together as single eluent is always preferable. Gradient elution is a powerful tool in achieving separation between closely eluting compounds or compounds having widely differing in polarities. The important feature of the gradient elution which makes it a powerful tool is that the polarity and ionic strength of the mobile phase can be changed (can be increased or decreased) during the run.

Selection of detector wavelength

Selection of detector wavelength is a critical step in finalization of the analytical method for impurities and degradants. Inject the impurity and API standard solutions into the chromatographic system with photodiode array detector and collect the spectra. Following are the detectors used for Spectroscopic analysis

UV Detector: An ultraviolet (UV) ray detector is a device which uses photoelectric cells to detect the presence of UV rays and the amounts in which they can be found. They are generally used to test lighting fixtures and electronics to discover the amounts of UV emitted before being sold to consumers. UV detector devices can also be used by consumers to find out how much UV radiation is in their homes, businesses, and other locations and sometimes where the rays are coming from.

PDA Detector: A photodiode array (PDA) is a linear array of discrete photodiodes on an integrated circuit (IC) chip. For spectroscopy it is placed at the image plane of a spectrometer to allow a range of wavelengths to be detected simultaneously. In this regard it can be thought of as

an electronic version of photographic film. Array detectors are especially useful for recording the full uv-vis absorption spectra of samples that are rapidly passing through a sample flow cell, such as in an HPLC detector.

PDAs work on the same principle as simple photovoltaic detectors.

ANALYTICAL METHOD VALIDATION AS PER GUIDELINES

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable to identification, control of impurities and assay procedures id included.[30]

1.	Accuracy	5.	Detection Limit
2.	Precision	6.	Quantification Limit
3.	Linearity and Range	7.	Ruggedness
4.	Specificity	8.	Robustness